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THE CHARACTERISTICS OF BACTERIA OF THE COLON TYPE FOUND IN BOVINE FECES *

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INTRODUCTION

In a former paper¹ from this laboratory giving the results of a study of bacteria of the colon group occurring in milk, three principal points were made: (1) The gas ratio and volume under uniform conditions are quite constant; (2) on the basis of the gas ratio and volume the collection of cultures under observation was divided into two distinct groups, one of which was probably a mixture of two or more varieties, while the other, giving a CO₂ to H₂ ratio of approximately 1.1, was distinct and homogeneous; (3) the fermentation of carbohydrates and alcohols is closely correlated with the gas ratio, indicating that this marks the lines of natural relationship within the group.

We found in the milk samples one group holding to the type with great uniformity and distinguished by the comparatively small volume of gas, a CO₂ to H₂ ratio of 1.1, and by a somewhat limited ability to ferment carbohydrates and alcohols. A considerable percentage of this group, however, fermented the resistant alcohol dulcitol. About 50 per cent. of the cultures isolated from milk gave a ratio varying from 1.5 to 2.8, a large volume of gas, and, in general, the ability to ferment all of the test substances used with the exception of dulcitol. A small number liquefied gelatin, a character apparently correlated with the fermentation of glycerin.

The low ratio group evidently included those members of the colon group usually designated as *Bacillus coli communis* and *Bacillus coli communior*, while in the high ratio group we may recognize *Bacillus lactis aërogenes*, *Bacillus acidi lactici* and possibly *Bacillus cloacae*.

In determining the sanitary quality of both water and milk, the origin of bacteria of the colon type and the directness with which they

* Received for publication February 3, 1914.

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1. *Jour. Infect. Dis.*, 1914, 14, p. 411.

are transmitted from their natural habitat to the water or the milk are matters of great importance. In previous work the difficulty of making subdivisions of the group which were not purely arbitrary, and consequently uncertain, has prevented the determination of the sources of the colon group in more than a very general way. With more promising methods of differentiation, we were encouraged to determine the habitat of the various varieties and especially to secure more complete information on our high-ratio group. With this end in view, a representative collection of gas formers of the colon type was isolated from cow feces. These cultures were studied, as before, on the basis of the gas formation as determined by exact methods, the formation of indol, the liquefaction of gelatin and the fermentation of a set of carbohydrates and alcohols. These results are tabulated to show the correlations which may indicate the separation of the group into naturally related divisions.

ORIGIN OF THE CULTURES

The 150 cultures studied in this series were obtained from seventeen samples of feces from as many different cows on the Dairy Division farm. A small part of the sample was transferred with a platinum loop to a water blank from which lactose-gelatin and asparagin agar plates were made. This latter medium, which has been tried extensively in this laboratory by Mr. Ayers, has been found particularly adapted to the isolation of the colon group. From the two sets of plates, typical colonies were transferred to dextrose broth tubes containing small inverted tubes. From those showing gas, after incubation at 30 C., five to ten were selected and replated. The tubes were selected so that the final cultures in each case came partly from the asparagin agar plates incubated at 30 C., and partly from the gelatin plates incubated at 20 C.

MORPHOLOGY

The morphology of all cultures was determined by the examination of stained preparations made from agar cultures incubated twenty-four hours at 30 C. Camera lucida drawings were made, using a combination which gave a magnification of 4,800 diameters on the drawing board. Typical cells, selected from a number of cultures, are shown in Figure 1. These do not include the very long thread-like cells which occur occasionally. There is a wide variation in both the size and the

form of the cell, but since these variations frequently occur within the limits of a single culture they are without varietal significance.

THE LIQUEFACTION OF GELATIN

As a rule, a culture liquefying gelatin is not included in the colon group although in the report of the committee of the American Public Health Association² the classification of this group provides for strains which liquefy gelatin. In our collection from milk we found a few cultures responding to this test. The cultures under consideration were tested by adding a few drops of broth culture to the surface of a gelatin tube, paraffining the plug to reduce evaporation, and measuring the liquefaction after 30 days at 20 C. None of this collection gave any evidence of liquefaction.

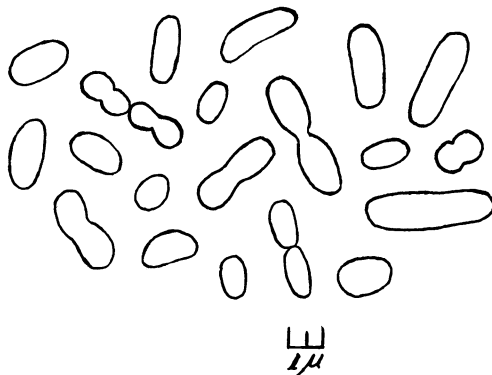


Fig. 1.—Morphology of typical cells.

THE INDOL TEST

Indol was determined by the method of Zipfel.³ The determination was made in a medium consisting of 1,000 c.c. water, 0.3 gm. of tryptophan and 5 gm. of K_2HPO_4 . Ten c.c. of this medium were used and the test made after two days' incubation at 30 C. The presence of indol was determined by adding drop by drop to each tube 1 c.c. of a 2 per cent. alcoholic solution of p-dimethylamidobenzaldehyd and concentrated hydrochloric acid. If indol was present, a violet color appeared. The medium was colorless and the reaction was sharp and clear. The results are given in Table 4. It will be noted that only

2. Standard Methods for the Examination of Water and Sewage, New York, 1912, p. 80.

3. *Centralbl. f. Bacteriol.*, Abt. 1, Orig., 1913, 67, p. 572; *Ibid.*, 67, p. 572; *Ibid.*, 1912, 63, p. 545.

one of the 150 cultures failed to give a positive reaction, and that this culture was sharply differentiated from the others by its high gas volume and ratio.

THE FERMENTATION OF CARBOHYDRATES AND ALCOHOLS ;
GAS FORMATION

Methods.—In the study of the gas production of these bacteria, the method of Keyes⁴ was again used, with the modifications described by Rogers, Clark and Davis.⁵ Essentially it consists in growing the bacteria in evacuated and sealed bulbs, in collecting the gas with mercury pumps and in analyzing the gas over mercury. Instead of the Topley pumps formerly used, two automatic Sprengel pumps were employed. To keep these efficiently dry, while evacuating the larger quantity of medium employed in the present series, it was found necessary to interpose a phosphorus pentoxid bulb between the fall tube of the pump and the sulphuric acid drying tube, described in our former paper. To render the Sprengel pumps available at all times for the collection of gas, we finally abandoned the practice of making the preliminary evacuation with these, and instead used a Geryck oil pump with a proper drying chain. While the preliminary evacuation could not be carried so near to completion by this means, no difference in the results could be detected. In all the evacuations the pressure was estimated by observing the nature of the discharge from an induction coil when passed through a small Plücker tube attached to the pump. Such discharges were compared at known pressures determined with a McLeod gauge, and so we are able to state that the gas pressure indicated in the pump at the conclusion of the preliminary evacuation was well below 0.06 mm., and that the gas pressure indicated at the close of the collection of the gas was considerably below 0.03 mm. As a matter of fact, the pressure of actual gas left in the bulbs at the close of the preliminary evacuation was probably somewhat lower than that indicated above because of the sweeping effect of the water vapor. There is, however, considerable difference of opinion among various authors concerning the effectiveness of this. We therefore prefer to be conservative. Furthermore, the pressure indicated at the close of the gas collection was to us simply an indication that the evacuation had reached a point where the amount of gas retained by the medium was too small to

4. *Jour. Med. Research*, 1909, 21, p. 69.

5. *Jour. Infect. Dis.*, 1914, 14, p. 411.

measure by our methods. We fully realize how nearly impossible it is to completely remove all the gas from a liquid by evacuation.

In our former series a medium made up as follows was employed:

"To 1,000 c.c. distilled water were added 4 gm. Liebig's beef extract and 10 gm. Witte's peptone. This was heated on a steam bath for twenty minutes, filtered, and the loss of water made up. The acidity of a sample was determined by titration with tenth normal sodium hydrate, using phenolphthalein as an indicator. The whole was then neutralized with normal sodium hydrate. After five minutes' heating the medium was again filtered, its reaction determined, and if 10 c.c. required more than 0.2 c.c. tenth normal sodium hydrate it was neutralized, heated, and again filtered. There were now added 1 per cent. dextrose (Kahlbaum) and 0.5 per cent. dibasic potassium phosphate (K_2HPO_4). After the solution of these was complete the medium was filtered and its volume at room temperature made up to 1,000 c.c."

In our present series the above medium was abandoned as unnecessarily complicated. The Liebig's extract was not only unnecessary for an abundant growth of the organisms we were considering, but we had reason to believe that its introduction makes a medium which is not accurately reproduceable. Furthermore, the neutralization with alkali, which is almost certain to contain some carbonate however small an amount, introduces a slight inconstant error in the determination of carbon dioxide; and it may also produce a less accurately reproduceable initial reaction in the medium than the method of preparation we will now describe.

The medium used in the present series was made up as follows:

Ten gm. Merk's "Highest Purity" dextrose, 10 gm. Witte's peptone, and 5 gm. Kahlbaum's dibasic potassium phosphate (K_2HPO_4) were added to 800 c.c. distilled water. This mixture was heated with occasional stirring, for twenty minutes over steam. It was then filtered through a Schleicher and Schull No. 588 folded filter. The filtrate was allowed to cool to 20 C. and then made up to 1,000 c.c. The substances were weighed with reasonable accuracy on a chemical balance, and all volumetric apparatus used was accurately calibrated. As soon as prepared, the medium, in accurately measured amounts, was apportioned in the previously cleaned, dried, and sterilized bulbs. It was then fractionally sterilized in steam on three successive days.

The quantity of this medium taken for each experiment was in most cases 10 c.c., and this was held in bulbs somewhat larger than those formerly used. But the old bulbs were still available and were used with 5 c.c. of medium. When this was done the data were calculated for 10 c.c. when incorporated in the tables. The manner of inoculation, and the methods of evacuation, sealing off, collecting the gas, and the analysis of the gas, are fully described in our former paper. It

TABLE 1
GAS PRODUCTION OF BACTERIA ISOLATED FROM COW FECES

Medium: 10 c.c., 1 per cent dextrose; 1 per cent peptone; $\frac{1}{2}$ per cent. K_2HPO_4 . Incubation temperature, 30 C. Incubation period, 7 days.

Gas Collected with Mercury Pumps

Organism	Total Volume in Cubic Centimeters	Percentage of CO_2	Percentage of H_2	Percentage of Residual Gas	Ratio CO_2/H_2	Organism	Total Volume in Cubic Centimeters	Percentage of CO_2	Percentage of H_2	Percentage of Residual Gas	Ratio CO_2/H_2
22hm	12.83	51.1	47.7	1.2	1.07	22jk	13.62	51.1	48.2	0.7	1.06
22hm	15.30	52.3	48.0	...	1.09	22jl	14.17	50.3	49.2	0.5	1.02
22hn	13.83	50.5	49.2	0.3	1.03	22jm	11.04	51.3	48.7	0.0	1.05
22ho	15.98	51.2	48.3	0.5	1.06	22jn	15.07	50.8	48.6	0.6	1.04
22hp	12.44	51.5	48.5	0.0	1.06	22jo	13.51	50.0	49.5	0.5	1.01
22hq	12.96	51.5	48.5	0.0	1.06	22jp	14.18	51.7	47.6	0.7	1.09
22hr	12.60	51.0	48.7	0.3	1.05	22jq	12.56	53.7	44.9	1.4	1.20
22hs	13.82	52.1	46.5	1.4	1.12	22jr	13.59	51.1	48.3	0.6	1.06
22ht	13.85	50.2	49.0	0.8	1.02	22js	12.50	51.0	48.7	0.3	1.05
22hu	12.25	50.1	48.5	1.4	1.03	22jt	14.21	51.1	48.5	0.4	1.05
22hu	14.98	52.4	47.2	0.4	1.11	22ju	14.03	53.0	46.5	0.5	1.14
22hu	13.43	49.6	49.4	1.0	1.00	22jv	14.35	50.2	49.5	0.3	1.01
22hv	13.56	51.3	48.2	0.5	1.06	22jw	13.08	52.6	46.6	0.8	1.13
22hw	16.80	51.2	48.6	0.2	1.05	22jx	14.54	50.1	49.1	0.8	1.02
22hx	15.08	50.5	49.1	0.4	1.03	22jy	15.50	51.2	47.6	1.2	1.08
22hy	13.24	52.1	47.1	0.8	1.11	22jz	13.72	51.0	48.7	0.3	1.05
22hy	10.88	51.2	47.7	1.1	1.07	22ka	13.66	52.1	46.9	1.0	1.11
22hz	16.10	50.4	47.6	2.0	1.06	22kb	14.40	50.2	49.4	0.4	1.02
22ia	13.90	49.9	49.6	0.5	1.00	22kc	12.48	51.0	47.8	1.2	1.07
22ia	15.82	51.5	47.9	0.6	1.07	22kf	12.71	51.0	48.8	0.2	1.05
22ib	13.24	50.2	49.6	0.2	1.01	22kg	14.78	51.4	47.4	1.2	1.09
22id	14.10	52.8	46.2	1.0	1.14	22kh	13.80	50.5	48.8	0.7	1.03
22ie	12.12	50.6	48.7	0.7	1.04	22ki	12.14	50.8	48.7	0.5	1.04
22ie	13.07	51.6	47.7	0.7	1.08	22kj	14.88	53.2	46.1	0.7	1.16
22if	12.26	51.1	48.0	0.9	1.06	22kl	14.71	50.4	49.1	0.5	1.03
22ij	14.39	50.6	48.4	1.0	1.04	22km	14.09	50.6	49.1	0.3	1.03
22ik	15.13	51.0	48.4	0.6	1.06	22kn	15.32	50.3	49.4	0.3	1.02
22il	13.74	52.3	47.0	0.7	1.11	22ko	10.72	49.6	49.2	1.2	1.01
22il	14.09	51.0	48.5	0.5	1.05	22kp	14.88	51.1	48.5	0.4	1.06
22im	14.52	51.2	47.4	1.4	1.08	22kq	13.58	50.4	49.0	0.6	1.03
22im	10.92	50.6	48.9	0.5	1.04	22kr	13.01	50.5	48.7	0.8	1.04
22in	13.29	51.9	47.8	0.3	1.09	22ks	14.46	51.7	47.6	0.7	1.09
22ip	12.35	51.3	47.8	0.9	1.07	22kt	15.28	51.0	48.6	0.4	1.05
22iq	14.04	52.1	44.9	3.0	1.16	22ku	14.24	51.8	47.2	1.0	1.10
22iq	14.04	52.2	47.3	0.5	1.10	22kv	14.94	51.2	48.9	...	1.05
22ir	12.69	49.6	49.5	0.9	1.00	22kw	14.37	50.3	49.3	0.4	1.02
22ir	14.51	49.8	49.7	0.5	1.00	22kw	18.40	50.3	48.6	1.1	1.04
22is	12.13	50.6	49.1	0.3	1.03	22kw	20.40	51.2	48.2	0.6	1.06
22it	12.36	51.9	48.1	0.0	1.08	22kx	12.95	50.8	48.3	0.9	1.05
22it	12.00	50.5	47.6	1.9	1.06	22ky	14.08	50.5	49.2	0.3	1.03
22iu	14.64	51.3	48.2	0.5	1.06	22kz	14.22	51.3	48.2	0.5	1.06
22iu	11.79	49.9	48.8	1.3	1.02	22la	16.02	50.4	49.1	0.5	1.03
22iv	14.36	51.1	48.7	0.2	1.05	22lc	14.24	50.4	49.0	0.6	1.03
22iw	13.81	50.0	49.9	0.1	1.00	22ld	12.68	49.9	49.6	0.5	1.01
22iw	14.15	50.4	48.8	0.8	1.03	22le	14.58	51.9	47.3	0.8	1.10
22ix	13.69	51.4	47.3	1.3	1.09	22lf	13.87	50.1	49.7	0.2	1.01
22iy	13.76	49.5	49.3	1.2	1.00	22lf	12.79	50.7	49.0	0.3	1.03
22iy	14.60	50.8	48.5	0.7	1.05	22lg	13.79	50.5	48.8	0.7	1.03
22iz	15.27	49.8	48.3	1.9	1.03	22lh	14.75	50.2	49.3	0.5	1.02
22ja	13.54	50.7	48.7	0.6	1.04	22li	15.50	51.9	47.0	1.1	1.10
22jb	12.25	50.5	48.9	0.6	1.03	22lj	17.95	51.9	47.6	0.5	1.09
22jc	14.80	51.8	47.6	0.6	1.09	22lk	13.89	50.7	48.7	0.6	1.04
22jd	14.40	50.1	49.3	0.6	1.02	22ll	14.43	50.9	48.8	0.3	1.04
22je	13.82	49.7	49.4	0.9	1.01	22lm	12.86	52.3	46.8	0.9	1.12
22je	12.96	50.5	48.5	1.0	1.04	22ln	12.58	49.2	50.2	0.6	0.98
22jf	14.11	52.5	47.1	0.4	1.12	22ln	13.23	50.5	49.5	0.0	1.02
22jf	14.39	51.3	48.0	0.7	1.07	22lo	13.72	52.8	45.6	1.6	1.16
22jg	13.46	52.6	46.7	0.7	1.13	22lo	14.33	53.1	46.3	0.6	1.15
22jh	13.99	51.1	48.2	0.7	1.06	22lp	15.96	52.1	47.0	0.9	1.11
22ji	12.85	50.9	48.1	1.0	1.06	22lq	15.02	50.2	49.1	0.7	1.02

TABLE 1—*Continued*

Organism	Total Volume in Cubic Centimeters	Percentage of CO ₂	Percentage of H ₂	Percentage of Residual Gas	Ratio CO ₂ /H ₂	Organism	Total Volume in Cubic Centimeters	Percentage of CO ₂	Percentage of H ₂	Percentage of Residual Gas	Ratio CO ₂ /H ₂
221r	15.26	52.6	47.1	0.3	1.12	22mn	18.50	51.3	48.1	0.6	1.07
221r	12.68	50.6	48.9	0.5	1.03	22mn	16.37	51.4	48.0	0.6	1.07
221s	14.87	49.5	49.1	1.4	1.01	22mo	13.46	50.5	48.8	0.7	1.03
221t	16.09	49.9	49.5	0.6	1.01	22mq	13.70	50.2	49.6	0.2	1.01
221u	14.11	50.6	49.1	0.3	1.03	22mr	16.25	51.5	47.9	0.6	1.07
221v	15.72	52.0	46.6	1.4	1.11	22ms	12.17	51.5	48.3	0.2	1.07
221w	15.54	51.9	48.0	0.1	1.08	22mt	12.94	51.2	48.7	0.1	1.05
221x	14.09	53.2	46.4	0.4	1.15	22mu	15.18	51.4	48.0	0.6	1.07
221x	15.40	50.4	49.4	0.2	1.02	22mw	12.56	50.8	48.7	0.5	1.04
221x	14.25	51.2	48.2	0.6	1.06	22my	14.30	49.8	49.2	1.0	1.01
221y	14.94	49.6	49.7	0.7	1.00	22my	15.03	50.8	48.8	0.4	1.04
221y	13.98	50.3	48.6	1.1	1.04	22mz	14.42	50.4	49.2	0.4	1.03
221z	17.10	49.7	49.7	0.6	1.00	22na	14.94	53.8	45.3	0.9	1.19
221z	15.07	49.9	49.5	0.6	1.01	22na	13.13	51.0	48.0	1.0	1.06
221z	13.19	49.7	49.7	0.6	1.00	22nb	12.80	51.2	47.7	1.1	1.08
22ma	13.10	52.4	46.4	1.2	1.13	22nc	13.02	51.4	47.8	0.8	1.08
22ma	13.73	50.8	49.2	0.0	1.03	22nd	13.14	50.7	48.9	0.4	1.04
22mb	14.93	50.8	48.5	0.7	1.05	22ne	13.10	51.2	48.1	0.7	1.07
22mc	13.83	52.4	46.8	0.8	1.12	22nf	14.66	51.0	48.6	0.4	1.05
22md	13.42	50.7	48.9	0.4	1.04	22ng	14.25	51.4	47.9	0.7	1.07
22me	14.42	52.3	46.9	0.8	1.12	22nh	14.92	53.9	45.8	0.3	1.18
22mf	16.30	51.5	47.9	0.6	1.08	22nh	17.52	52.1	47.9	0.0	1.09
22mg	15.21	50.2	49.5	0.3	1.01	22ni	12.27	49.7	48.7	1.6	1.02
22mh	13.20	51.0	48.3	0.7	1.06	22ni	13.80	51.2	48.3	0.5	1.06
22mi	15.12	52.0	47.2	0.8	1.10	22nj	14.04	51.1	48.1	0.8	1.06
22mj	14.42	50.1	49.4	0.5	1.01	22nk	14.54	51.9	47.9	0.2	1.08
22mk	13.60	52.5	45.9	1.6	1.14	22nl	14.83	50.8	49.2	0.0	1.03
22mk	14.14	52.7	46.7	0.6	1.13	22nm	15.59	51.0	48.7	0.3	1.05
22ml	15.20	50.6	48.9	0.5	1.03	22nn	13.00	50.9	48.1	1.0	1.06
22mm	13.43	51.1	48.3	0.6	1.06	22np	14.64	52.5	47.1	0.4	1.11
22mn	12.50	53.4	45.6	1.0	1.17	22nq	14.12	50.4	49.1	0.5	1.03

should be noted that all gas volumes have been reduced to dryness and to normal temperature and pressure.

Discussion of Analyses.—The analyses show a very remarkable agreement in the gas production of the organisms. The significance of this will be discussed later. A preliminary discussion of the analyses themselves will prove interesting.

In Table 1 are assembled 182 analyses of the gas produced by 149 of the cultures isolated as described on page 100. In the first column of this table will be found the laboratory designation of the culture; in the next column, the total gas produced per 10 c.c. of medium; in the three succeeding columns the composition of this gas in percentages of CO₂, of H₂, and residual gas. The residual gas is doubtless nitrogen. No hydrocarbons have been detected. In the last column will be found the ratio CO₂ to H₂. For the experiments recorded in this table the period of incubation was 7 days, a safe but perhaps unnecessarily long period. The incubation temperature was 30 C.

As in our former series, so also in this, it was considered unnecessary to make duplicate determinations when the results of an individual analysis were close to the mean of all. But several of these determinations, which in one way or another were found to be farthest from the average, were repeated. Almost without exception this duplication placed the resultant mean distinctly closer to the total average. It should be noted, however, that this practice of repeating only the less concordant experiments makes the duplications appear less harmonious than would have been the case had all determinations been duplicated. We have excluded from Table 1 five analyses which were incomplete because of definitely known, though slight, errors in the determination of hydrogen. The percentages of carbon dioxide in these cases were 50, 49.5, 53.2, 50.6 and 51. We have also taken the liberty of excluding two analyses, one of 22kx and one of 22ht, both of which seemed to agree with their duplicates so far as they could be judged, but in both of which the data were incongruous. In one of these a leak during analysis was suspected. During the other analysis a sudden interruption occurred, and it is believed that the readings were incorrectly recorded. We have also withheld from the table the analyses of two cultures which we will discuss later.

The agreement in Table 1 is evidently sufficiently close to justify our using the arithmetical mean rather than the mode. The averages are as follows:

Total volume, 14.09; CO_2 , 51.1 per cent.; H_2 , 48.2 per cent.; residual gas, 0.7 per cent.; ratio CO_2 to H_2 , 1.06. If the average ratio, 1.06, is compared with the mode estimated for what we called the color group in our former paper, it will be seen that our present average is somewhat lower. This may be attributed in large measure to the medium. To compare the two media we picked at random five cultures of the present series and grew them in bulbs containing 10 c.c. of the old medium. The two sets of results are compared in Table 2.

These differences, though slight, are distinct and should be kept in mind when comparing the present and the old series. The nature of the agreement in Table 1 becomes clearer when the volumes of carbon dioxide and of hydrogen are plotted as they have been in Figure 2. In this figure the values of the two gases for the analyses summarized in Table 1 all fall close to the line of the equation $1.06x = y$, or in other terms $\text{CO}_2/\text{H}_2 = 1.06$; 1.06 is the average ratio of the group.

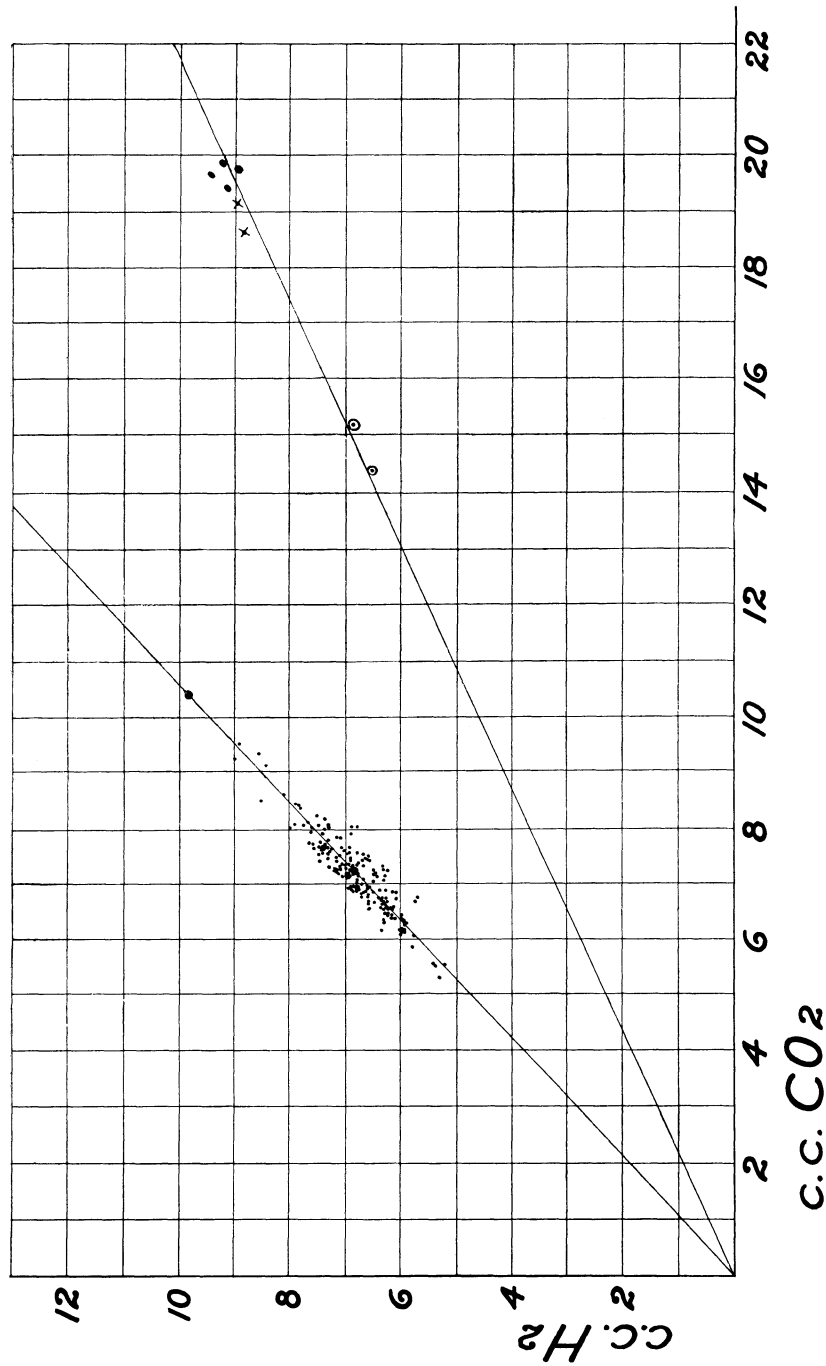


Fig. 2.—Gas determinations on all cultures. Small dots (.) represent all cultures from feces which approximate the equation $CO_2:H_2=1.06$.
 ○ 22h from milk; X 22h from bovine feces.

A glance at this region visualizes the point made in the paper by Rogers, Clark and Davis,⁶ that such variations as occur in this group are essentially differences in the extent of the fermentation, differences which appear in the total volume of gas rather than in the ratio of the constituent gases. In a few instances one culture will apparently persist in giving a larger total volume than another; but whether we may legitimately draw any distinction between such cases is a question.

It is well to lay emphasis on this variation in total gas, for it is doubtless the source of considerable confusion when the gas is determined with the Smith or Durham tube. As Frieber⁷ has ably shown, two cultures which differ only in the amount of gas produced but not in

TABLE 2
COMPARISON OF THE GAS PRODUCTION OF SEVERAL BACTERIA WHEN GROWN IN VACUO ON THE OLD AND ON THE NEW MEDIA

Medium	Organism	Total Gas in Cubic Centimeters	Percentage of CO ₂	Percentage of H ₂	Percentage of N ₂	Ratio CO ₂ /H ₂
New	22hz	16.10	50.4	47.6	2.0	1.06
Old	22hz	16.84	54.5	45.0	0.5	1.21
New	22kl	14.71	50.4	49.1	0.5	1.03
Old	22kl	17.87	53.4	46.2	0.4	1.16
New	22lw	15.54	51.9	48.0	0.1	1.08
Old	22lw	16.23	53.6	45.7	0.7	1.17
New	22lx	14.58	51.6	48.0	0.4	1.08*
Old	22lx	16.89	54.3	45.2	0.5	1.20
New	22mz	14.42	50.4	49.2	0.4	1.03
Old	22mz	16.81	53.7	45.7	0.6	1.17

* Averages.

the ratio of the constituent gases, will give totally different ratios when these are determined by any method which does not correct for the dissolved CO₂. Frieber was unfortunately not aware of the work of Keyes and Gillespie⁸ in which it was shown that the presence of oxygen alters the ratio to a large degree and from a cause not yet fully determined. In view of this fact and the other sources of error in the old methods of making the gas test, it would be futile to attempt any estimation of the error. Nevertheless, we suspect that several of our organisms which gave the larger volumes of gas would not have been identified with *Bacillus coli* had they been in the hands of those who place confidence in the ordinary methods of analysis, and who rely on a low ratio of CO₂ to H₂ as characteristic of *Bacillus coli communis*.

6. *Jour. Infect. Dis.*, 1914, 14, p. 411.

7. *Centralbl. f. Bakteriolog.*, Abt. 1, Orig., 1913, 69, p. 437.

8. *Jour. Biol. Chem.*, 1912, 13, p. 305.

Among the organisms isolated by the method described on page 100 are two, the gas determinations of which demand special comment.

Early in the series 22ic gave the following data: Total gas, 22.53; CO_2 , 68.5 per cent.; H_2 , 31.1 per cent.; residual gas, 0.4 per cent.; ratio CO_2/H_2 , 2.2. This placed ic distinctly in that second group of gas producers described in our former paper. When later in the work duplicate determinations were made, ic surprised us by giving the total gas and ratio which are typical of the low-ratio group. Since only one such abrupt and distinct change has been noted in some one thousand analyses which we have made in the past three years, and since the former case was traced to a mistake in lettering the culture,⁹ a similar mistake was suspected in this instance. We therefore at once looked over the stock cultures and found that one of two tubes containing the ii culture had been labeled with no dot over the second i and might easily have been taken for ic. We were at the first prejudiced against this explanation, because, without consulting the records, we supposed that ii had been found to produce no growth in our anaërobic bulbs. In this our records showed us to be mistaken, and when ii came to be tried it was found to give the volume and ratio observed with what had been supposed to be a culture of ic. These characteristics were given continuously by ii, while ic continued to give the lower volume and ratio characteristic of the *Bacillus coli* group. We were unfortunate in this instance in that we had no dried sample of the original cultures with which to check our results, as we had in the case of cw, recorded in our former paper. But it is certain that the cultures of ic and ii had not been mixed or contaminated, since repetition of their fermentations and the observance of their cultural characteristics identified the cultures of each as the same organism originally isolated. We are therefore confident that an undotted i was read as a c. This conclusion was proposed and agreed on as possible by three of us at a time when we were prejudiced against it by the false supposition that ii gave no gas.

We therefore feel justified in taking the later data as characteristic of each of these cultures, and in saying that ii is the one and only culture among the 150 isolated as described on page 100, which gives a gas ratio distinct from those shown in Table 1. In Table 3 are summarized all the analyses made of the gas produced by ic and ii. These analyses of ii place it quite distinctly in that second group of gas pro-

9. *Jour. Infect. Dis.*, 1914, 14, p. 411.

ducers described in our former paper. We have shown the position in Figure 2 of the above values for CO_2 and H_2 obtained with ii by the four dots in the region $\text{CO}_2 = 19-20$, $\text{H}_2 = 9-10$. Near this are two determinations with 22s, an organism which fell in the second group of our former series. These are indicated by crossed dots. Closer to the origin are two circled dots indicating the position of 22aj, an organism which, in the former series on the more complex medium, fell in most but not all cases beyond 22s and in what we described as a provisional third group.

It is interesting to note that all three of these organisms give data which fall rather close to the line of the equation CO_2 to $\text{H}_2 = 2.17$ in which 2.17 is the average ratio of the eight determinations.

On these facts further comment will be made in a future paper; but at this point we wish to call attention to a remark made in our

TABLE 3
ANALYSES OF GAS PRODUCED BY IC AND II

Organism	Total Gas in Cubic Centimeters	Percentage CO_2	Percentage H_2	Percentage of Residual Gas	Ratio CO_2/H_2
ic?	22.53	68.5	31.1	0.4	2.20
ic	14.92	52.7	46.7	0.6	1.13
ic	15.58	52.7	46.8	0.5	1.13
ic	16.55	51.7	47.7	0.6	1.08
ic	15.57	51.0	48.7	0.3	1.05
ii	29.10	67.5	32.1	0.4	2.10
ii	28.65	67.8	31.7	0.5	2.14
ii	29.08	68.4	31.4	0.2	2.18
ii	28.70	68.8	30.9	0.3	2.23

former paper to the effect that we judged the discrepancies then found were due more to inaccuracies of method than to variations in the physiologic functions of the cultures. It certainly is true that there is closer agreement among the present data of the colon group, and while we should be cautious in drawing conclusions from the few determinations made with the high-ratio organisms, the eight harmonize in a remarkable way. We are inclined to attribute these facts to the simplification of the medium.

ACID FORMATION

Determinations were made of the amount of acid formed by all cultures in broth containing dextrose, saccharose, lactose, raffinose, starch, inulin, mannite, glycerin, adonite and dulcite. The broth was

made according to the following formula: Water, 100 gm.; peptone, 1 gm.; beef extract, 4 gm.; dibasic sodium phosphate, 0.5 gm.; test substance, 1 gm.

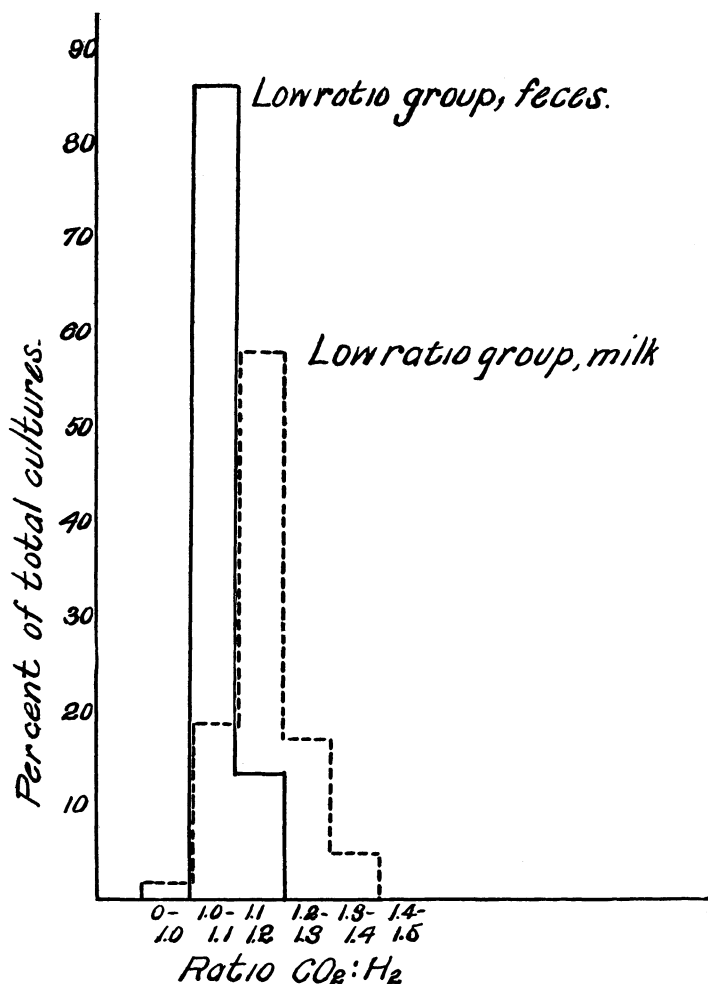


Fig. 3.—Frequency of occurrence of $\text{CO}_2:\text{H}_2$ ratios.

This was sterilized in the Arnold on three successive days. The cultures were incubated seven days and after they were steamed to remove the excess of carbon dioxide they were titrated against $n/20$ normal sodium hydrate. The results, which are given in Table 4, are

TABLE 4

FERMENTATIVE REACTIONS FOR ALL CULTURES, ACIDITY EXPRESSED AS PER CENT NORMAL ACID

Culture	Indol	Dextrose	Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite
hm	+	6.51	4.65	4.98	5.40	.00	Alk	4.75	1.30	Alk	2.30
hn	++	5.63	4.73	5.00	5.05	Alk	Alk	5.13	2.85	Alk	2.75
ho	++	5.58	Alk	5.30	Alk	Alk	Alk	4.95	2.60	Alk	Alk
hp	++	5.88	4.63	5.08	5.05	Alk	Alk	3.85	3.60	Alk	.25
hq	++	5.35	4.73	4.55	4.75	Alk	Alk	4.75	3.15	Alk	1.50
hr	++	5.52	4.75	5.40	4.95	Alk	Alk	5.05	3.60	Alk	1.10
hs	++	5.23	5.20	5.10	4.83	1.88	Alk	4.55	3.45	Alk	1.75
ht	++	5.76	Alk	5.00	Alk	Alk	Alk	4.65	3.40	1.85	Alk
hu	++	5.61	3.70	5.55	5.20	.76	Alk	4.75	3.10	Alk	3.20
hv	++	4.88	4.57	4.63	5.50	Alk	Alk	4.80	3.10	Alk	3.50
hw	++	2.63	Alk	4.85	Alk	Alk	Alk	4.85	1.60	1.80	Alk
hx	++	5.28	.85	4.60	5.05	Alk	Alk	4.45	3.40	Alk	1.50
hy	++	5.66	4.20	5.20	4.60	Alk	Alk	4.93	3.75	Alk	1.75
hz	++	5.58	4.25	4.75	5.25	Alk	Alk	4.80	3.05	Alk	3.00
ia	++	5.45	Alk	5.07	Alk	Alk	Alk	4.50	4.25	1.85	Alk
ib	++	4.91	4.68	4.40	5.25	Alk	Alk	4.85	2.45	Alk	2.75
ic	++	5.28	5.60	5.20	5.48	Alk	Alk	4.37	1.50	2.10	Alk
id	++	5.63	4.78	5.10	6.35	.90	Alk	4.52	3.00	Alk	2.30
ie	++	5.68	3.80	4.93	4.34	Alk	Alk	5.20	3.25	Alk	.70
if	++	8.78	3.70	4.95	3.70	Alk	Alk	4.75	2.70	Alk	1.75
ii	++	3.60	3.35	5.80	4.12	2.05	Alk	2.50	.05	1.40	1.40
ij	++	5.68	4.20	5.30	4.45	Alk	Alk	4.67	4.55	Alk	1.65
ik	++	5.51	5.00	4.97	5.17	Alk	Alk	4.45	2.95	Alk	3.50
il	++	5.44	4.13	5.15	4.00	Alk	Alk	4.65	2.35	Alk	3.05
im	++	5.98	4.18	5.15	4.92	Alk	Alk	4.75	3.70	Alk	2.00
in	++	5.93	4.25	4.35	4.75	Alk	Alk	4.85	3.10	Alk	1.30
ip	++	5.58	4.15	5.10	5.28	Alk	Alk	4.85	3.80	Alk	1.45
iq	++	5.60	5.47	5.22	4.85	Alk	Alk	5.13	1.90	Alk	1.50
ir	++	6.00	4.64	5.40	5.58	Alk	Alk	4.85	2.65	Alk	3.05
is	++	4.43	4.00	4.55	5.16	Alk	Alk	4.95	.80	Alk	2.60
it	++	5.73	4.15	5.80	5.47	Alk	Alk	4.70	.60	Alk	.65
iu	++	5.50	3.90	4.95	5.15	Alk	Alk	4.58	3.25	Alk	2.00
iv	++	5.73	Alk	4.83	Alk	1.03	Alk	4.73	1.80	2.25	Alk
iw	++	5.65	Alk	4.50	Alk	Alk	Alk	4.30	3.40	1.60	Alk
ix	++	5.58	Alk	5.03	Alk	Alk	Alk	4.95	1.40	1.80	Alk
iy	++	5.43	4.90	3.70	5.35	Alk	Alk	5.17	2.60	Alk	3.32
iz	++	5.73	4.70	5.90	5.00	Alk	Alk	5.05	1.15	Alk	2.70
ja	++	4.58	Alk	4.15	Alk	Alk	Alk	4.75	2.95	1.50	Alk
jb	++	5.63	Alk	5.15	.88	1.08	Alk	4.00	3.00	1.60	Alk
jc	++	5.88	5.00	5.35	5.50	Alk	Alk	4.55	2.55	Alk	2.15
jd	++	5.65	Alk	5.00	Alk	Alk	Alk	4.80	3.10	1.40	Alk
je	++	5.19	4.60	5.05	6.05	.93	Alk	4.85	1.25	Alk	2.15
jf	++	5.48	4.15	4.65	4.70	3.43	Alk	4.50	3.25	Alk	3.15
jg	++	5.66	4.25	5.05	5.45	4.75	Alk	4.60	2.15	Alk	2.35
jh	++	5.58	Alk	4.50	1.25	Alk	Alk	4.85	3.35	Alk	1.20
ji	++	5.58	4.10	5.45	5.15	3.23	Alk	4.55	3.25	Alk	3.45
jk	++	5.81	Alk	5.55	Alk	2.33	Alk	4.65	3.65	Alk	2.90
jl	++	5.73	Alk	5.05	Alk	2.88	Alk	4.70	3.35	1.95	Alk
jm	++	5.96	5.10	5.45	5.20	1.78	Alk	4.55	3.55	Alk	2.45
jn	++	5.38	Alk	2.45	Alk	Alk	Alk	5.10	2.85	1.50	Alk
jo	++	5.71	5.65	5.25	5.45	Alk	Alk	4.90	.85	Alk	2.40
jp	++	5.88	5.00	5.60	5.75	Alk	Alk	4.95	3.25	Alk	1.70
jq	++	8.08	4.00	5.60	5.70	Alk	Alk	5.00	2.20	Alk	2.70
jr	++	5.76	4.35	4.95	5.65	Alk	Alk	5.05	3.25	Alk	2.05
js	++	5.88	Alk	4.70	Alk	Alk	Alk	4.45	3.15	Alk	2.15
jt	++	5.78	3.95	5.00	5.05	Alk	Alk	4.45	3.85	Alk	2.25
ju	++	5.68	4.95	5.15	5.60	Alk	Alk	4.60	3.45	Alk	2.45
jv	++	5.53	4.35	5.40	5.80	Alk	Alk	5.10	4.15	Alk	3.20
jw	++	5.76	4.05	5.20	5.60	Alk	Alk	4.45	4.55	Alk	1.25
jx	++	5.38	4.30	5.30	5.65	Alk	Alk	3.95	2.95	Alk	1.90
jy	++	5.83	5.15	5.40	5.40	Alk	Alk	5.10	2.95	Alk	2.85
jz	++	5.58	Alk	5.25	Alk	1.68	Alk	4.80	3.15	1.80	Alk
ka	++	5.28	Alk	5.30	Alk	Alk	Alk	5.60	3.15	2.20	Alk

CHARACTERISTICS OF COLON BACTERIA IN BOVINE FECES 113

TABLE 4—*Continued*

Culture	Indol	Dextrose	Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcitol
kb	++	5.90	4.50	4.95	5.60	Alk	Alk	4.90	3.75	Alk	3.05
kc	++	5.83	4.40	5.15	5.60	Alk	Alk	4.80	3.65	Alk	3.60
kf	++	6.08	3.10	5.10	5.50	Alk	Alk	4.80	2.75	Alk	1.15
kg	++	5.63	Alk	5.05	Alk	Alk	Alk	4.80	3.05	Alk	1.75
kh	++	5.54	4.20	5.80	5.45	Alk	Alk	4.75	1.55	Alk	Alk
ki	++	6.08	Alk	5.20	Alk	Alk	Alk	4.80	3.25	Alk	2.75
kl	++	5.73	Alk	5.30	Alk	Alk	Alk	4.80	2.95	Alk	2.95
km	++	5.43	Alk	5.25	Alk	Alk	Alk	4.50	3.45	Alk	1.70
kn	++	5.53	Alk	5.70	Alk	Alk	Alk	4.80	1.35	Alk	3.00
ko	++	5.58	Alk	5.40	Alk	Alk	Alk	4.65	2.75	Alk	2.25
kp	++	6.18	Alk	4.85	Alk	Alk	Alk	5.50	4.15	Alk	3.95
kq	++	5.58	Alk	5.30	1.05	Alk	Alk	5.00	2.05	Alk	Alk
kr	++	5.08	Alk	5.00	Alk	Alk	Alk	4.60	2.35	Alk	2.95
ks	++	5.63	4.55	5.40	5.40	Alk	Alk	5.05	3.25	Alk	3.75
kt	++	5.58	Alk	4.80	.00	Alk	Alk	5.15	2.15	Alk	2.80
ku	++	5.83	Alk	5.05	Alk	Alk	Alk	4.85	3.05	Alk	3.85
kv	++	5.10	3.90	4.30	4.80	Alk	Alk	4.40	2.50	Alk	3.50
kx	++	6.00	Alk	6.30	Alk	Alk	Alk	4.95	2.95	Alk	Alk
ky	++	5.38	3.60	5.50	4.15	Alk	Alk	2.80	3.25	Alk	1.50
kz	++	5.08	Alk	5.30	Alk	Alk	Alk	3.90	3.35	Alk	2.95
la	++	5.85	Alk	4.77	Alk	Alk	Alk	4.95	2.15	Alk	1.20
lc	++	5.63	Alk	4.75	2.80	Alk	Alk	4.55	3.25	Alk	2.30
ld	++	5.70	Alk	4.15	Alk	Alk	Alk	5.00	3.65	Alk	1.70
le	++	5.43	4.80	5.75	5.70	Alk	Alk	5.10	4.25	Alk	3.70
lf	++	5.58	4.26	5.35	5.10	Alk	Alk	4.95	2.95	Alk	1.75
lg	++	5.76	4.15	5.15	4.90	4.33	Alk	4.40	4.05	Alk	3.45
lh	++	5.68	Alk	5.00	Alk	3.18	Alk	4.90	3.15	Alk	1.65
li	++	5.58	4.30	5.90	4.90	Alk	Alk	4.15	2.70	Alk	2.80
lj	++	5.68	3.90	3.45	6.15	Alk	Alk	5.30	4.05	Alk	3.40
lk	++	5.55	3.80	5.30	4.80	Alk	Alk	4.00	3.55	Alk	3.50
ll	++	5.48	Alk	2.95	Alk	Alk	Alk	4.20	2.45	Alk	1.60
lm	++	5.68	2.90	6.50	4.40	3.25	Alk	4.70	3.30	Alk	3.50
ln	++	5.15	4.10	5.65	5.35	Alk	Alk	4.90	4.05	Alk	3.30
lo	++	5.18	4.60	5.30	5.25	Alk	Alk	4.75	3.35	Alk	2.60
lp	++	5.66	3.45	5.35	5.85	Alk	Alk	4.65	4.05	Alk	2.15
lq	++	4.28	Alk	5.10	.55	Alk	Alk	3.50	1.75	Alk	.60
lr	++	5.68	3.70	5.30	4.80	Alk	Alk	4.40	3.40	Alk	2.55
ls	++	5.38	4.15	5.65	4.85	Alk	Alk	4.85	2.75	Alk	3.45
lt	++	5.55	Alk	5.60	Alk	Alk	Alk	4.55	2.05	Alk	1.90
lu	++	5.58	Alk	5.15	Alk	Alk	Alk	4.60	2.75	Alk	2.25
lv	++	5.40	3.70	5.50	5.15	Alk	Alk	4.60	3.65	Alk	4.00
lw	++	5.68	Alk	4.80	Alk	Alk	Alk	4.60	2.95	Alk	1.60
lx	++	5.31	4.45	5.40	5.15	Alk	Alk	4.65	3.15	Alk	3.85
ly	++	5.63	Alk	5.05	Alk	.38	Alk	4.60	3.75	Alk	1.60
lz	++	3.88	Alk	5.80	Alk	Alk	Alk	3.90	1.45	Alk	Alk
ma	++	6.03	Alk	5.50	Alk	Alk	Alk	4.95	1.95	Alk	Alk
mb	++	5.08	4.00	6.50	4.15	.30	Alk	4.60	2.90	Alk	2.75
mc	++	5.73	Alk	4.70	Alk	Alk	Alk	5.05	3.65	Alk	Alk
md	++	4.98	Alk	4.80	Alk	Alk	Alk	4.40	3.25	Alk	2.65
me	++	5.33	Alk	5.20	Alk	.83	Alk	4.60	3.55	Alk	2.60
mf	++	6.78	Alk	4.80	Alk	Alk	Alk	3.90	3.00	Alk	1.85
mg	++	5.73	Alk	4.55	Alk	Alk	Alk	5.15	2.95	Alk	2.80
mh	++	5.83	3.85	5.20	5.85	Alk	Alk	4.50	3.45	Alk	2.15
mi	++	5.68	4.55	4.75	5.65	Alk	Alk	4.00	2.05	Alk	3.20
mj	++	5.65	0.10	4.35	0.20	Alk	Alk	4.60	1.75	Alk	2.10
mk	++	5.78	3.50	5.20	4.35	Alk	Alk	4.20	3.00	Alk	2.45
ml	++	5.63	Alk	5.00	Alk	Alk	Alk	4.85	3.25	Alk	1.75
mm	++	6.08	3.90	3.90	4.80	Alk	Alk	4.20	3.55	Alk	2.50
mn	++	5.63	Alk	4.75	Alk	Alk	Alk	4.95	2.25	Alk	2.30
mo	++	5.88	3.70	5.60	3.65	Alk	Alk	2.70	3.40	Alk	1.70
mq	++	5.83	4.05	5.25	5.25	1.23	Alk	4.70	3.05	Alk	1.55
mr	++	5.76	3.60	5.30	4.85	Alk	Alk	3.90	3.85	Alk	3.00
ms	++	5.68	2.40	5.20	4.60	Alk	Alk	4.70	3.05	Alk	3.85
mt	++	5.58	Alk	4.30	Alk	Alk	Alk	2.80	2.30	Alk	Alk
mu	++	5.48	4.00	5.20	5.55	Alk	Alk	4.70	4.35	Alk	1.80
	++	5.78	Alk	4.75	Alk	Alk	Alk	5.35	3.65	Alk	Alk
	++	5.71	Alk	3.25	Alk	Alk	Alk	3.90	2.25	Alk	Alk

TABLE 4—*Continued*

Culture	Indol	Dextrose	Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite
mw	+	5.68	4.20	5.20	5.35	Alk	Alk	4.95	3.05	Alk	2.70
my	++	5.43	4.90	5.45	5.05	Alk	Alk	5.00	3.50	Alk	2.80
mz	++	7.88	3.50	6.00	5.90	Alk	Alk	3.80	2.85	Alk	3.35
na	++	6.78	4.25	5.40	5.65	Alk	Alk	4.85	3.65	Alk	.95
nb	++	5.68	3.70	5.50	5.15	1.23	Alk	4.45	4.25	Alk	2.75
nc	++	6.53	3.70	5.15	4.95	Alk	Alk	4.70	3.25	Alk	2.50
nd	++	5.58	0.15	5.05	0.35	Alk	Alk	4.10	3.25	Alk	2.80
ne	++	5.58	4.25	5.20	5.30	Alk	Alk	5.55	3.95	Alk	.40
nf	++	5.58	4.80	5.50	5.40	Alk	Alk	4.45	3.05	Alk	Alk
ng	++	5.68	Alk	5.20	.60	Alk	Alk	5.10	3.95	Alk	2.65
nh	++	5.73	3.75	4.90	5.25	0.63	Alk	6.05	3.15	1.55	2.05
ni	++	5.68	0.20	5.00	Alk	Alk	Alk	4.40	2.85	Alk	2.48
nj	++	5.50	Alk	5.15	Alk	.93	Alk	4.30	4.05	Alk	2.65
nk	++	6.08	Alk	6.30	Alk	Alk	Alk	3.30	2.90	Alk	1.00
nl	++	5.58	5.10	5.05	5.20	Alk	Alk	4.55	1.15	Alk	2.10
nm	++	5.88	Alk	5.20	1.20	Alk	Alk	4.70	2.70	Alk	2.70
nn	++	5.83	4.20	5.80	5.35	Alk	Alk	4.85	3.15	Alk	.90
np	++	5.88	4.45	5.35	5.20	Alk	Alk	4.55	2.05	Alk	.00
nq	+	6.25	Alk	6.30	Alk	Alk	Alk	4.65	2.50	Alk	Alk

expressed as percentage of normal acid. There was only a small variation in the acidity produced by individual cultures in the different test substances. The acidity is used, therefore, only as a means of determining the ability of the culture to ferment the sugar. Any reaction of 1 per cent. is considered positive.

CORRELATION OF RESULTS

In our earlier paper the marked correlation between the gas ratio of 1.1 to 1.3 and certain fermentative reactions was pointed out. The close agreement of the $\text{CO}_2 : \text{H}_2$ ratio of the feces cultures with that of low-ratio group of the milk collection has already been indicated. If the ratios are plotted on the frequency of occurrence basis, as given in Table 5, Figure 3 is obtained, which brings out very clearly the great uniformity in the results from such a large collection. For comparison, there is plotted on the same figure the milk culture frequency curve, recalculated on the basis of percentage of total low-ratio cultures. Making allowance for the difference due to the more complicated medium used with the earlier collection, we find that, so far as the gas ratio shows, the two sets of cultures are practically identical. Table 6 shows that while the amount of gas produced is subject to more variation than the gas ratio, the variation is confined to comparatively narrow limits, and when accurately determined the volume of gas from a

given amount of standard medium may be taken as characteristic of a type. In Table 7 is arranged the percentage of the total number of cultures which ferment the various test substances. In this table and those which follow the one culture giving a high ratio is excluded. In order to make these results more comprehensible they are plotted in Figure 4. By this arrangement the percentage of cultures giving a

TABLE 5
DISTRIBUTION OF CULTURES ON BASIS OF GAS RATIO

Gas Ratio	0.9-1.0	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4
No. of cultures	0	126	29	1	0
Percentage of cultures	0	85.7	13.6	0.7	0

TABLE 6
DISTRIBUTION OF CULTURES ON BASIS OF VOLUME OF GAS

Cubic Centimeters of Gas	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21
No. of cultures	0	1	27	40	50	20	6	1	1	1	0
Percentage of cultures..	0	0.7	18.4	27.2	34.0	13.6	4.1	0.7	0.7	0.7	0

TABLE 7
PHYSIOLOGICAL REACTIONS OF ALL CULTURES
(Percentage of Positive and Negative Reactions)

	Gelatin	Indol	Dextrose	Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite
No. of positive reactions.	0	148	149	88	149	93	16	0	149	145	31	102
Percentage of positive reactions	00	99.3	100	59.1	100	62.4	10.7	00	100	97.3	20.8	68.2
No. of negative reactions	149	1	0	61	0	56	133	149	0	4	118	47
Percentage of negative reactions	100	0.7	00	40.9	00	37.6	89.3	100	00	2.7	79.2	31.8

positive reaction are placed on the left of the median line, while those giving a negative reaction are on the right. This shows that the type culture always forms indol from tryptophan, fails to liquefy gelatin, ferments dextrose, lactose, mannite and glycerin, frequently ferments saccharose, raffinose and dulcite, but only rarely ferments starch, inulin or adonite. The lack of uniformity in these reactions suggests that by further subdivision, varieties would be found in which the percentage of cultures which give either a positive or negative reaction with cer-

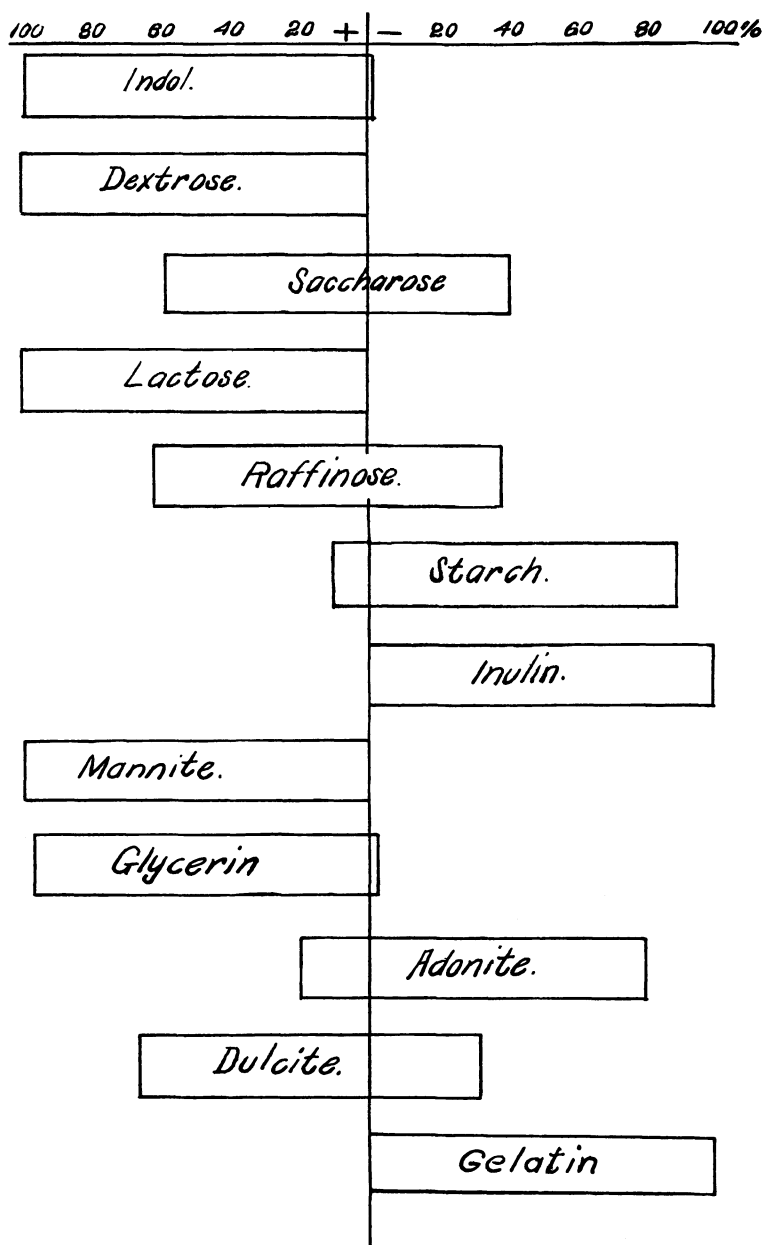


Fig. 4.—Showing ability to act on test substances. Positive results plotted to left of median line, negative results to right.

tain sugars would be materially reduced. A group answering these requirements may be obtained by dividing these cultures on the basis of saccharose fermentations. This gives us the two sharply defined groups shown in Table 8 and Figure 5. There is a positive correlation between the fermentation of saccharose, raffinose and dulcitol, and a negative correlation between saccharose and adonitol. This correlation between saccharose and raffinose fermentation has already been noted by Howe¹⁰ in his studies of the colon-like organisms isolated from human feces. On the one hand, we have a group the members of which ferment dextrose, saccharose, lactose, raffinose, mannitol, glycerin and usually dulcitol, while they almost invariably fail to utilize starch, inulin

TABLE 8
CORRELATION BETWEEN FERMENTATION OF SACCHAROSE AND OTHER TEST SUBSTANCES

	Dextrose	Lactose	Raffinose	Starch	Inulin	Mannitol	Glycerin	Adonitol	Dulcitol
Saccharose + (88)—									
No. of positive reactions	88	88	88	10	0	88	84	3	77
Percentage of positive reactions	100	100	100	11.4	00	100	95.4	3.4	87.5
No. of negative reactions	0	0	0	78	88	0	4	85	11
Percentage of negative reactions	00	00	00	88.6	100	00	4.6	96.6	12.5
Saccharose — (61)—									
No. of positive reactions	61	61	5	6	0	61	61	28	25
Percentage of positive reactions	100	100	8.2	9.8	00	100	100	45.9	41.0
No. of negative reactions	0	0	56	55	61	0	0	33	36
Percentage of negative reactions	00	00	91.8	90.2	100	00	00	54.1	59.0

or adonitol. On the other hand, we have a group fermenting dextrose, lactose, mannitol and glycerin, but which almost always fails to ferment saccharose, raffinose, starch and inulin, but not infrequently ferments adonitol and dulcitol.

The collection obtained from milk came from a wide range of sources and could not be expected to give so uniform results as we have obtained from the cultures coming from a single source. The striking agreement, however, which is obtained when these two collections are classified on the same basis is shown in Figure 5, and is

10. *Science*, 1912, 35, p. 225.

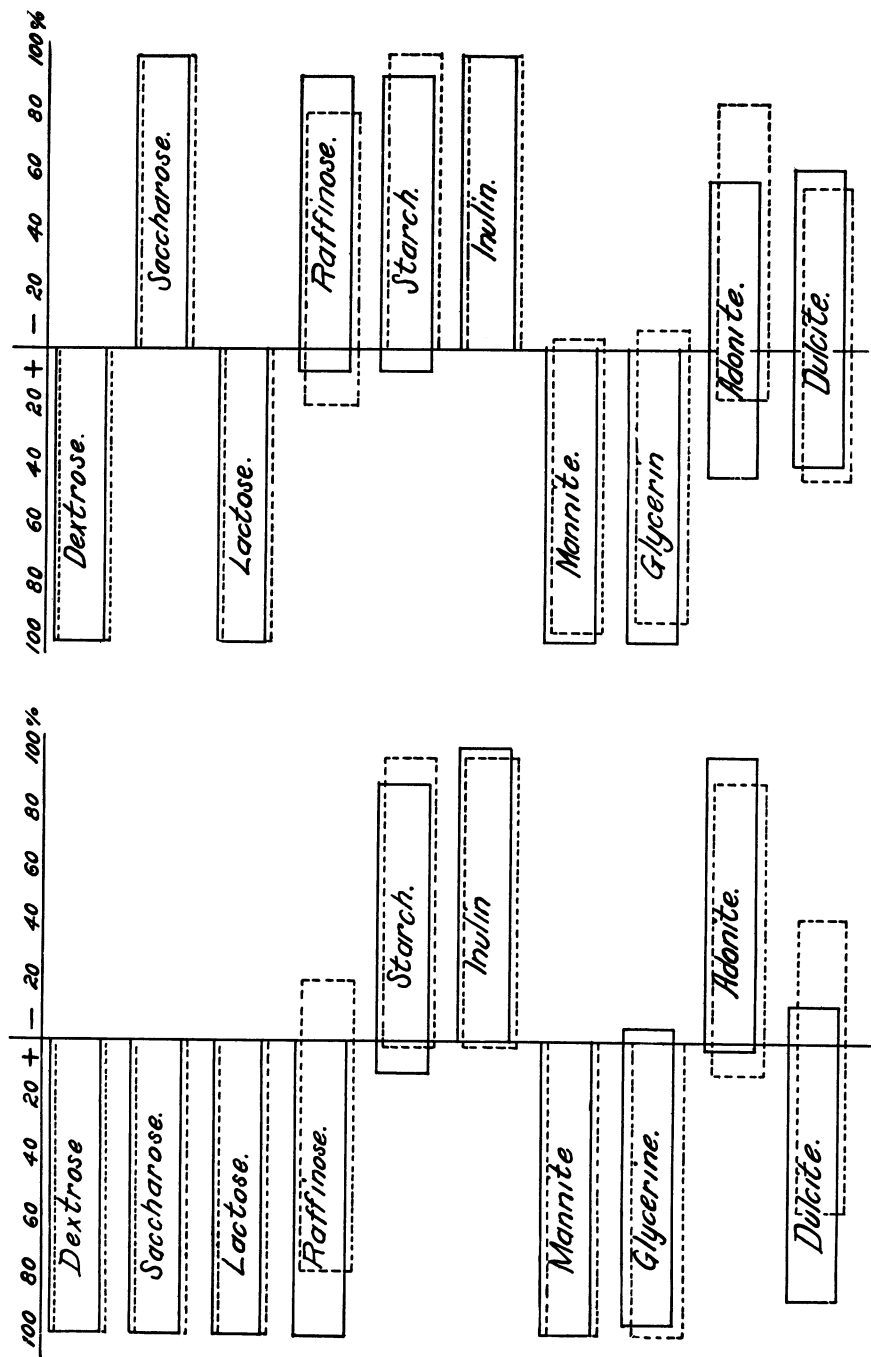


Fig. 5.—Two subgroups of the low-ratio groups. Solid lines represent cultures from feces, broken lines cultures from milk.

strong evidence that the groups, as we have arranged them, are legitimate. Howe does not publish his complete data, and while it is possible that his collection obtained from the digestive tract of man may have obtained some high-ratio cultures, it is evident that about half of his cultures were identical with our saccharose-raffinose-dulcitate fermenting group. It is probable that the remaining cultures may be classified with our non-saccharose fermenting group. The classification proposed by Jackson¹¹ and adopted by the American Public Health Association¹² includes under *Bacillus coli* only cultures of the colon type which ferment dulcitate, and divides these into *Bacillus coli communior*, which ferments saccharose, and *Bacilli coli communis*, which fails to ferment saccharose. There is no evidence, however, that these groups have any basis other than convenience in identification. We would suggest, therefore, that the low-ratio group, which as a whole has very definite characters and limitations, be designated as *Bacillus coli*, and that the varietal name *Bacillus coli communior* be applied to the saccharose-raffinose fermenting subgroup, and *Bacillus coli communis* to the group which fails to ferment these sugars. It should be noted that two individual cultures do not agree in all particulars with the classification outlined. One, jq, forms a pigment on agar and gives an exceptionally high acid formation in dextrose broth. Otherwise it does not differ from the typical cultures. The only other exception was ii, which belongs in the high-ratio group, and which was excluded from the correlation tables.

THE ABSENCE OF THE HIGH-RATIO GROUP

In the collection of gas-forming bacteria obtained from milk, 48 per cent. were sharply differentiated from the low-gas-ratio group by the high-gas ratio and certain fermentative reactions. We have seen, on the contrary, that only one culture of our collection from bovine feces could be placed with the high-ratio group. This may possibly be due to the fact that the high-ratio group occurred in such small numbers, in comparison with the low-ratio group, that only in one case were we able to pick off a sufficient number of colonies to secure a representative of the high-ratio group. It may also be possible that the methods of isolation were selective and tended to exclude one group. This is very improbable, as two temperatures and two media of quite different com-

11. *Jour. Am. Pub. Health Assn.*, 1911, 1, p. 930.

12. *Standard Methods for the Examination of Water and Sewage*, New York, 1912, p. 80.

position were used. Either group grows readily on gelatin, and all members of the high-ratio group which were tested grew well on the asparagin agar. A special effort was made to isolate from feces cultures which would give the characteristic high-ratio reaction. It was observed in the earlier results that adonite was fermented by 55 per cent. of the high-ratio group, but by only 15 per cent. of the low-ratio group. It would be expected, therefore, that if cultures were selected on the basis of the fermentation of adonite the chance of their belonging to the high-ratio group would be increased. Four samples of cow

TABLE 9
ANALYSES OF THE GAS PRODUCED FROM DEXTROSE BY BACTERIA OF COW FECES WHICH
FERMENT ADONITE

Organism	Total Gas in Cubic Centimeters	Percentage CO ₂	Percentage H ₂	Percentage Residual Gas	Ratio CO ₂ /H ₂
22ns	13.41	50.9	48.5	0.6	1.05
22nt	11.04	52.6	46.5	0.9	1.13
22nu	13.42	51.3	48.0	0.7	1.07
22nv	10.03	51.9	47.6	0.5	1.09
22nw	11.41	50.9	48.7	0.4	1.05
22nx	13.18	50.9	48.7	0.4	1.05
22ny	13.26	51.8	47.9	0.3	1.08
22nz	14.07	52.3	47.6	0.1	1.10

TABLE 10
GAS RECOVERED FROM 10 C.C. MEDIUM INOCULATED DIRECTLY WITH COW FECES AND
INCUBATED AT 30 C.

Sample	Total Gas	Percentage CO ₂	Percentage H ₂	Ratio CO ₂ /H ₂
5	2.01	51	47	1.07
5	5.48	52	47	1.09
6	2.19	52	45	1.16
8	4.02	52	46	1.13
8	4.10	52	46	1.13

feces were added to tubes of milk and of adonite broth, and incubated at 30 C. After twenty-four hours these tubes were plated on adonite-litmus agar and the acid colonies which developed were transferred to adonite-agar stabs. All of these stabs gave abundant gas.

Table 9 shows that none of the cultures so obtained could belong to the high-ratio group. A still more conclusive test was made by direct inoculation of five vacuum-fermentation bulbs with samples of cow feces. While it is probable that the growth of both types of the colon group would be suppressed by other bacteria, particularly by the strep-

tococci, we would expect to find, if the high-rate group was present, some evidence of an increasing proportion of carbon dioxide.

The following analyses of the gas produced in 10 c.c. of Broth 1, while they do show that a suppression of the production has taken place, do not indicate the presence of any high-ratio gas producers in the cow feces.

The failure of these special methods to give any evidence of the presence of high-ratio bacteria makes it reasonably safe to conclude that members of this group occur only rarely in the bovine intestinal tract.

CONCLUSIONS

These results give additional evidence of the constancy of bacterial reactions when they are determined under uniform conditions and by exact methods. The $\text{CO}_2 : \text{H}_2$ ratio, which has been generally discarded as valueless on account of its great variation, varied from 0.98 to 1.20 in determinations on 149 cultures. This is, no doubt, due to the fact that we have under consideration a closely related natural family which, when it ferments carbohydrates, is able to do so by one set of reactions only. These reactions are repeated by each individual of the family with mathematical accuracy, and variations from the theoretical end-products are due to lack of refinements in our methods, or to extraneous factors which use up some of the end products. The same conditions which have produced a family bringing about these particular reactions have also impressed on the group the ability to utilize certain carbohydrates and alcohols as sources of food or energy. It is evident that through some peculiarities in the protoplasmic molecule, the ability to ferment one substance carried with it the ability to utilize certain other substances also, and in some cases the inability to ferment others. However, the ability or inability to ferment certain substances is not so basic a function as the power of producing from these substances a definite chemical reaction with constant end-products. Consequently, we have more variation in the former function, a variation which produces subfamilies giving in common the same kind and proportion of end-products, but differing in the materials from which they are able to obtain them. The minor variations in the volume of gas produced under definite conditions are probably due to the variation exhibited by different cultures in ability to withstand the acid produced by their own activities. Consequently the evolution of gas ceases earlier in some cultures than in others. There is, however, a close correlation between

the gas volume and $\text{CO}_2:\text{H}_2$ ratio, and the volume is evidently a distinctive characteristic of some families. These families are doubtless produced under the long-continued influence of uniform conditions, and when we begin to select our cultures from a definite habitat it becomes easy to establish natural groups with remarkably constant and typical characters. Thus these results show that the bovine intestine is the habitat of two distinct but closely related varieties of the colon group, just as an earlier paper¹³ from this laboratory has shown that a distinct variety of the streptococci can be obtained from the same source.

The negative results have an interest second only to the establishment of the limitations of *Bacillus coli* group as it occurs in bovine feces. Of 125 cultures isolated from milk and answering all of the usual presumptive tests for *Bacillus coli*, almost one-half possessed characters which differentiated them sharply from the type found almost exclusively in bovine feces. Only one of 150 cultures from bovine feces gave reactions which identified it with the high-ratio group, which made up nearly 50 per cent. of the milk cultures. These results are surprising as there is no apparent reason why the high-ratio group, which, in our opinion, is identical with the so-called *aërogenes-lactici acidii* group, should not grow in the intestinal tract with its relative, *Bacillus coli*. Nevertheless, our efforts to isolate more than a single culture have so far failed, and the obvious conclusion, assuming the correctness of our results, is that the habitat of the high-ratio group must be sought elsewhere. An attempt will be made in this laboratory to obtain a definite answer to this question.

SUMMARY

Previous work on a collection of the colon type from milk demonstrated that the gas ratio and volume are constant under uniform conditions; that, on the basis of the gas ratio and volume, the cultures may be divided into two distinct groups; and that the correlation of the fermentative ability with the gas ratio makes this distinction sharply defined.

This paper records the results of a similar study on 150 cultures isolated from bovine feces. None of these cultures liquefied gelatin and all but one formed indol from tryptophan. By the use of a simple medium and exact methods of analysis, it was found that in 149 cul-

13. Rogers and Dahlberg, *Jour. Agricult. Research*, 1914, 1, p. 491.

tures the $\text{CO}_2:\text{H}_2$ ratio varied only from 0.98 to 1.20. One culture only gave a ratio identifying it with the high-ratio group, which made up 48 per cent. of the milk series.

The 149 low-ratio (0.98-1.20) cultures were readily divided into two groups, one of which fermented dextrose, saccharose, lactose, raffinose, mannite, glycerin and dulcite, but almost invariably failed to ferment starch, inulin and adonite, while the second group fermented adonite and dulcite and failed to ferment saccharose, raffinose, starch and inulin.

These groups agree almost perfectly with two groups which may be formed from the low ratio cultures isolated from milk. Special methods failed to give evidence, with the exception of the single culture mentioned, of the presence in bovine feces of the high-ratio group, which made up about one-half of the milk collection.